Pharmacology, Biochemistry and Behavior 100 (2011) 165–173

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Maternal prolactin inhibition at the end of lactation affects learning/memory and anxiety-like behaviors but not novelty-seeking in adult rat progeny

Mabel C. Fraga [a,b], Egberto G. Moura [a], Juliana Oliveira Silva [a,b], Isabela Teixeira Bonomo [a], Cláudio C. Filgueiras [b], Yael Abreu-Villaça [b], Magna C.F. Passos [a], Patrícia C. Lisboa [a], Alex C. Manhães [b,*]

[a] Laboratório de Fisiologia Endócrina, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Brazil

[b] Laboratório de Neurofisiologia, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 14 February 2011
Received in revised form 5 July 2011
Accepted 7 July 2011
Available online 18 July 2011

Keywords:
Hypoprolactinemia
Early weaning
Developmental plasticity
Behavior
Rats

ABSTRACT

Maternal hypoprolactinemia at the end of lactation in rats reduces milk production and is associated with offspring's malnutrition. Since malnutrition during development is also known to have long lasting effects on cognition and emotion, in the present study we tested the hypothesis that maternal hypoprolactinemia, induced by bromocriptine treatment, at the end of the lactating period affects memory/learning, novelty-seeking and anxiety-like behaviors in adult male Wistar rats using, respectively, the radial arm water maze (RAWM), the hole board (HB) arena and the elevated plus-maze (EPM). We also analyzed serum corticosterone and thyroid hormone levels at postnatal day (PN) 21. Lactating dams were treated with bromocriptine (BRO, 1 mg twice a day, inhibiting prolactin) or saline from PN19 to 21 (the last 3 days of lactation). BRO offspring had hypercorticosteronemia and hypothyroidism at PN21. In the RAWM, reductions in latency observed in CON rats were initially more accentuated than in BRO ones. By the end of the testing period, latencies became similar between groups. No difference was observed between groups regarding the number of nose-pokes in the HB. In the EPM, BRO rats stayed less time in and had fewer entries into the open-arms than CON ones. This pattern of results indicates that maternal bromocriptine treatment at the end of the lactating period results in poorer memory/learning performance and in higher levels of anxiety-like behavior in the adult offspring, demonstrating that even a relatively short period of malnutrition during development can have long lasting detrimental effects regarding cognition and emotion.

1. Introduction

Epidemiological and experimental studies have associated physiological alterations at adulthood with adverse conditions early in life, such as malnutrition, hormonal changes and other stressful events that are considered imprinting factors (De Moura et al., 2008; Plagemann and Harder, 2005; Ravelli et al., 1976). This phenomenon, which was initially denominated programming (Barker, 2003) and now is also known as developmental plasticity (Gluckman and Hanson, 2007), has been linked to obesity, diabetes, dyslipidemia, cardiovascular diseases (Armitage et al., 2008) and vulnerability to psychopathological disorders such as anxiety and depression (Charney and Bremner, 1999; Darnaudéry and Maccari, 2008). Several studies in animal models and humans have already shown that not only metabolic alterations but also cognitive and behavioral ones can be programmed (Molina et al., 1987; Kar et al., 2008; Fraga-Marques et al., 2009, 2010).

Undernutrition during early life results in permanent impairments in cognitive functions such as executive function, attention, working memory and visual perception (Kar et al., 2008; Levitsky and Strupp, 1995). Furthermore, several studies assessing the effects of breast-feeding have shown that children who are bottle-fed as babies present lower scores on intelligence tests than those who were breastfed later in life (Lucas et al., 1998; Rodgers, 1978). Since women are becoming progressively more active as working force, for many new mothers the return to work following maternity leave is a significant barrier to the continuation of breastfeeding (Galtry, 1997). Thus, experimental models of shortened lactation may be useful to understand the long lasting effects of a reduced period of breastfeeding on the behavioral, hormonal and nutritional statuses of the offspring.

Maternal hypoprolactinemia in rats can be induced by protein malnutrition of dams during lactation and is associated with reduced

* Corresponding author at: Laboratório de Neurofisiologia, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Av. Prof. Manuel de Abreu 444, 5 andar, Vila Isabel, Rio de Janeiro, RJ 20550-170, Brazil. Tel.: +55 21 2868 8195; fax: +55 21 2868 8029.

E-mail addresses: amanhaes@uerj.br, ac_manhaes@yahoo.com.br (A.C. Manhães).

0091-3057/
doi:10.1016/j.pbb.2011.07.007

milk production (Lisboa et al., 2006; Passos et al., 2000). Milk production can also be suppressed by treating lactating dams with bromocriptine (BRO) (Shah et al., 1988). Bromocriptine is an agonist of the type 2 dopaminergic receptor that, when administered to the dams, inhibits prolactin (PRL) synthesis in the pituitary gland (Shah et al., 1988). We have previously shown (Bonomo et al., 2005) that the offspring of dams that were BRO-injected during the last 3 days of lactation became malnourished during this period and that this procedure programmed the progeny, at adulthood, for obesity, hyperleptinemia, resistance to the anorexigenic effects of leptin (Bonomo et al., 2007), hypothyroidism (Bonomo et al., 2008), dyslipidemia, insulin resistance, hypercorticosteronemia and higher total catecholamine in adrenal medulla (De Moura et al., 2009). In spite of the fact that a considerable amount of information is available regarding metabolic and hormonal programming as a result of malnutrition at the end of lactation, none has been provided regarding the function of the nervous system. In this regard, the aim of the present study was to evaluate whether maternal PRL inhibition by the administration of BRO to dams during the late lactating period (from postnatal day 19 to 21) also results in behavioral changes at adulthood by assessing memory/learning, novelty-seeking and anxiety-like behaviors in male adult rat offspring.

2. Methods

2.1. Animal treatment

All experiments were carried out under institutional approval (CEA/187/2007) in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* (Publication no. 85-23, revised 1985) as adopted and promulgated by the National Institutes of Health and in accordance with Brazilian Law (Lei Arouca, Law #11.794, October 8th, 2008) (Marques et al., 2009).

Adult Wistar rats were kept in a temperature-controlled room on a 12-h light/12-h dark cycle (lights on at 7:00 am). Three-month-old virgin female rats were placed with male rats in a 2:1 ratio. After mating, pregnant rats were placed in individual cages. Access to food and water was *ad libitum*. At birth, (postnatal day 1 — PN1), all litters were culled to six male pups so as to maximize lactation performance (Fischbeck and Rasmussen, 1987; Passos et al., 2000). Litters that did not have at least six male pups were discarded. The anogenital distance was used to differentiate females from males and all experimental animals were accurately sexed. Sixteen lactating rats were separated into the following groups: 1) BRO — i.p. injected with 1 mg bromo-*a*-ergocriptine (Novartis, SP, Brazil), twice a day, during the last 3 days of lactation (PN19 to PN21) or 2) CON — which received i.p. injections of saline during the same period (Bonomo et al., 2005). After weaning (PN21), siblings were kept together throughout the experimental period (cage size: 39 cm long × 32 cm wide × 14 cm high) since single housing may influence behavior (Valzelli and Garattini, 1972). BRO and CON offspring had access to commercial diet and water *ad libitum*.

2.2. Body weight

During lactation, the body weights of the offspring were measured at PN1, PN4, PN8, PN12, PN16, PN18, PN19, PN20 and PN21. After PN21, body weight was monitored every fourth day until PN180.

2.3. Hormones

Serum corticosterone level was measured in 4 offspring (selected from different litters) and 5 dams of each group at PN21. After decapitation, which was carried out between 9:00 to 12:00 am, blood samples were collected and centrifuged (1000 × *g*, 4 °C, 20 min) to obtain serum, which was individually kept at −20 °C. All measurements were performed in one assay. Serum corticosterone level was measured by specific murine RIA kit (ImmuChemTM 125I, double antibody, ICN Biomedicals, Inc, USA). The assay sensitivity was 25 ng/ml and the intra-assay variation was 7.1%.

Total serum triiodothyronine (T3), thyroxine (T4) and TSH were determined by RIA in 8 BRO and 8 CON offspring (selected from different litters) at PN21. We used commercial kits (ICN pharmaceuticals, Inc., Costa Mesa, CA, USA) in which control standard curves diluted in iodothyronine-free rat serum (charcoal treated) were used. The intra-assay variation coefficient for T3 was 5.3%, with 25 ng/dl as the lower limit of detection, and for T4 the values were 3.3% and 2 μg/dl respectively. Serum TSH levels were measured using a kit for rat TSH supplied by the NIH (Boston, MA, USA) and data were expressed in terms of the reference preparation provided (RP-3). The intra-assay variation was 0.6% and the sensitivity limit was 0.18 ng/ml.

2.4. Behavioral testing

From PN172 to PN184, 40 BRO and 37 CON rats underwent behavioral testing. The following tests were used: 1) radial arm water maze (RAWM); 2) hole board arena (HB); and 3) elevated plus-maze (EPM). All testing sessions were performed between 2:00 and 6:00 pm in a sound attenuated room. A CON testing was always followed by a BRO one and *vice versa*. All tests were videotaped and the behavior was analyzed using the video images of the tests.

Memory/learning was assessed by testing rats at PN172 in the RAWM, which is shaped like an asterisk and consists of eight swim paths (arms: 29 cm long × 13 cm wide × 40 cm high) extending out of an open central area (41 cm diameter). The RAWM was filled with water (26 ± 1 °C) to a depth of 34 cm so that rats could not touch their feet or tails while swimming on the surface. An escape platform (8 cm long × 10 cm wide) was placed at the end of one of the arms, 1 cm below the surface. Non-toxic white paint was used to make the water opaque, hiding the platform. Several extra-maze cues were present in the testing room and their positions remained fixed throughout the entire experiment. A test began by placing the animal facing away from the arm that had the escape platform. Animals were tested for 5 consecutive days, 4 trials per day (inter-trial interval: 15 min). Animals were allowed 2 min per trial to explore the maze and find the hidden platform. If they failed to find the platform in a given trial during their allotted time, they were gently guided to it and allowed to stay on top of it for 20 s. The escape platform was not moved from its initial position during the first 4 days. In a sample of 28 BRO and 19 CON offspring, we also carried out a probe trial on the 5th day: The platform was placed in the arm opposite to the one in which it was located during the previous 4 days. The following variables were used to assess performance in the RAWM (Fraga-Marques et al., 2009, 2010; Jarrard, 1993): 1) Latency to find the hidden platform on each trial of each testing day (LAT); 2) Latency to first arm entry (LATARM; 3) Number of first entries into any arm which didn't have the platform (reference memory — RM); 4) Number of re-entries into the arm that had the platform (working memory correct errors — WMC); 5) Number of re-entries into any arm that did not have the platform (working memory incorrect errors — WMI). The pool was cleaned (bedding residue and fecal boli were removed) and the water thoroughly mixed between tests. The water was completely changed every day.

Novelty-seeking behavior was assessed by testing rats at PN180-181 in HB, which has the shape of a rectangular enclosed arena (80 cm long × 60 cm wide × 40 cm high). The base of the arena has 16 uniformly spaced holes (4 cm diameter) that the animals can explore. Animals were placed in the arena facing one of the walls and were allowed 10 min to explore. The number of nose-pokes (head-dips) was used as a measure of exploring activity (Abreu-

Villaça et al., 2006; Fraga-Marques et al., 2009, 2010). The number of rearings and grooming events (both indicating motor activity) was also used as ethological measures (Carola et al., 2002). The arena was cleaned with paper towels soaked in 50% ethanol and dried before each test.

Anxiety-like behavior was assessed by testing rats at PN182–184 in the EPM, which is shaped like a plus sign and consists of two "open" (no walls, 50 cm long × 10 cm wide) and two "closed" (50 cm long × 10 cm wide × 40 cm high) arms (OA and CA respectively), arranged perpendicularly, and elevated 50 cm above the floor. The test began with the animal being placed on the center of the equipment facing an open arm. Each test lasted 10 min. The total time spent in and the number of entries into each kind of arm and central area of the maze was recorded. The percentage of time spent in the open arms (%Time OA: the time spent in open arms divided by time spent in open + closed arms) and the percentage of open arms entries (%Entries OA: the number of entries in open arms divided by number of entries in open + closed arms) was also calculated (Manhães et al., 2008; Fraga-Marques et al., 2009, 2010). The number of closed arms entries (Entries CA) was used as a measure of activity. An entry was counted whenever the animal crossed with all four paws into an arm or into the central area. Increased Time OA, %Time OA, Entries OA or %Entries OA are indicative of a reduced anxiety state in the EPM (Fraga-Marques et al., 2009, 2010; Handley and McBlane, 1993). In addition, the percentage of time spent in the center of the maze (%Time CN: time spent in center divided by total time) was used as an independent measure of decision making (Manhães et al., 2008). Furthermore, ethologically derived parameters were also assessed in the EPM. These included rearing, number of closed arm returns, head dipping, grooming, and stretched attend posture (Johnson and Rodgers, 1996). The EPM was cleaned with paper towels soaked in 50% ethanol and dried before each trial.

2.5. Data analysis

Kolmogorov–Smirnov one sample tests (K–S) were used to assess the normality of the distributions of each of the variables. Significance is assumed at the level of $P<0.05$. For parametric distributions, data are compiled as means and standard errors of the means, while non-parametric data are compiled as medians and quartiles. A repeated measures analysis of variance (rANOVA) was used to analyze body weight throughout the experiment. TREATMENT (bromocriptine or control) was used as the between-subjects factor and DAY was considered the within-subjects factor. Student *t*-tests were used to compare serum corticosterone, T3, T4 and TSH levels between groups. Differences between the CON and BRO groups regarding latency to find the hidden platform (LAT) in the RAWM were analyzed by means of a repeated measures analysis of variance (rANOVA). DAY and TRIAL were considered the within-subjects factors. TREATMENT (bromocriptine or control) was used as the between-subjects factor. As for the other RAWM variables, LATARM, RM and WMI were analyzed using a multivariate ANOVA (mANOVA), while the WMC was analyzed using the Mann–Whitney test (M–W). The number of nose pokes in the HB was analyzed by means of a rANOVA (INTERVAL: within-subjects factor; TREATMENT: between-subjects factor). HB ethological data were analyzed by means of a multivariate ANOVA (mANOVA–TREATMENT: between-subjects factor). The EPM data Entries OA, %Entries OA, Time OA, and %Time OA were analyzed by means of the Mann–Whitney Test (M–W). Entries OA + CA, %Time CN, Return to CA and the other ethological data were analyzed by means of a mANOVA. (TREATMENT: between-subjects factor). Whenever the sphericity assumptions appeared to be violated (Mauchly's test) in the rANOVAs, an adjustment to the numerator and denominator degrees of freedom was made by using parameter ε (Huynh and Feldt, 1976). Lower-order ANOVAs and the Fisher Protected Least Square Difference test were used post hoc.

3. Results

3.1. Body weight

As indicated in Table 1, during the period of maternal bromocriptine treatment (PN19–PN21), BRO offspring presented significantly reduced body weight (7.7% reduction at PN21 for example) when compared to CON ones. After the treatment period, no significant differences were observed between groups up to the 3rd postnatal month. At PN180, BRO offspring were significantly heavier (+9.3%) than CON ones.

3.2. Hormones

Corticosterone levels of BRO dams at the end of lactation (PN21) were not significantly different than that of CON ones (*t*-test: $P>0.10$; BRO: 188 ± 32 ng/ml, CON: 278 ± 40 ng/ml). However, BRO offspring had significantly higher serum corticosterone levels than CON ones (*t*-test: $P=0.023$; BRO: 191 ± 19 ng/ml; CON: 88 ± 23 ng/ml). T3 levels were significantly reduced in BRO animals when compared to CON ones (*t*-test: $P=0.004$; BRO: 70 ± 3 ng/dl; CON: 86 ± 10 ng/dl), while no differences were observed between groups regarding T4 levels (*t*-test: $P>0.10$; BRO: 3.3 ± 0.8 μg/dl; CON: 3.3 ± 0.6 μg/dl). BRO animals also had significantly lower TSH levels than CON ones (*t*-test: $P=0.016$; BRO: 0.24 ± 0.02 ng/ml; CON: 0.32 ± 0.01 ng/ml).

3.3. Behavior

Fig. 1A shows that BRO animals presented a less accentuated improvement in performance (LAT) from the 1st to the 4th day than CON ones (rANOVA–TREATMENT × DAY interaction: $P=0.001$). This finding can be explained by the fact that while a significant reduction in latency can be observed from the 1st to the 2nd day in CON rats (FPLSD: $P=0.002$), no differences were observed in BRO ones. Furthermore, the fact that BRO rats were significantly faster (mANOVA: $P=0.028$) than CON ones in the 1st day and slower (mANOVA: $P=0.026$) in the 2nd helps to explain the observed interaction. No differences were observed regarding the 3rd, 4th and 5th (probe trial) days. As expected, the change in position of the platform resulted in increased latencies in both groups (FPLSD—4th *vs*. 5th day: $P<0.001$). The observed differences in latency in the 1st and 2nd days are associated with differences in RM (Fig. 1C) and WMI errors (Fig. 1D). In both cases, BRO animals presented a better performance than CON ones in the 1st day, and a worst one in the second. No differences were observed between groups regarding LATARM (irrespective of the position of the platform) (Fig. 1B) and the number of WMC errors. In the latter case, the median for both groups was zero with an interquartile range also of zero.

Maternal hypoprolactinemia had no effect (rANOVA: $P>0.10$) on the total number of nose pokes made by the adult offspring in the HB, as indicated in Fig. 2A. However, during the first time interval (initial 2.5 min), BRO animals had a tendency (rANOVA: $P=0.055$) to make less nose pokes than CON ones (Fig. 2B). No differences between groups were observed regarding the number of grooming (Fig. 2C) and rearing events (Fig. 2D).

As depicted in Fig. 3, BRO rats presented significantly reduced absolute and relative time spent in the open arms (M–W—Time OA: $P=0.015$; %Time OA: $P=0.009$, respectively) as well as a significant reduction in absolute number of entries into the open arms (M–W—Entries OA: $P=0.021$) when compared to the CON ones (Fig. 3A–C). BRO rats also presented reduced: Entries CA (mANOVA: $P<0.001$; Fig. 3F) and %Time Center (mANOVA: $P=0.002$; Fig. 3E). Regarding the ethological data, the only measure for which a significant difference was observed was the Closed Arm Returns (mANOVA: $P=0.002$).

Table 1
Body weight of the offspring throughout the experiment. Postnatal day 19 is the first day of bromocriptine treatment.

	Postnatal day										
Group	0	8	16	18	19	20	21	45	89	133	180
Control	6.8 ± 0.1	20.4 ± 0.3	38.3 ± 0.8	46.9 ± 1.0	50.4 ± 1.1 PS	53.1 ± 1.1*	55.5 ± 1.3**	183.7 ± 6.0	326.7 ± 7.9	377.5 ± 8.1***	397.4 ± 8.9***
Bromocriptine	6.5 ± 0.1	20.4 ± 0.6	38.3 ± 0.4	45.0 ± 0.7	47.9 ± 0.8	50.1 ± 0.7	51.2 ± 0.6	196.1 ± 4.0	345.8 ± 11.3	414.3 ± 16.4	438.2 ± 20.9

Values are means ± S.E.M, PS $0.10 \geq P \geq 0.05$.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

4. Discussion

4.1. Maternal bromocriptine treatment and changes in offspring's body weight and hormonal levels

In a previous paper (De Moura et al., 2009), we described somatic, metabolic and hormonal changes at different ages of the siblings of rats used in the present study, as well as data concerning serum PRL levels of the dams at weaning. We demonstrated that bromocriptine treatment significantly reduced PRL levels in the dams (−95%), but did not affect offspring levels at PN21, as expected (Bonomo et al., 2005; De Moura et al., 2009). In the present study, we confirm previous results (De Moura et al., 2009) indicating that treatment of dams with bromocriptine has deleterious effects on body weight gain of the offspring from PN19 to PN21, in spite of the fact that solid foods are already found in the offspring stomach during this period (Gomendio et al., 1995). It was also confirmed that BRO rats became heavier than CON ones at adulthood.

In general, fasting increases glucocorticoid levels (Ahima et al., 1996), which is consistent with our observation of higher corticosterone levels in BRO pups at PN21. This higher concentration in the offspring, but not in the dams, would seem to indicate that the former were stressed by the period of malnourishment while the dams were relatively unaffected by treatment with bromocriptine. Corticosterone is also known to affect thyroid function at several levels. For example, it inhibits the TRH–TSH–thyroid axis and the type 1 deiodinase in the liver, which converts T4 to T3 (Haugen, 2009). Furthermore, fasting also directly affects serum thyroid hormone concentrations (Moura et al., 1987). The aforementioned findings are consistent with our observations of lowered serum TSH and T3 levels. Interestingly, T4 levels did not vary as a function of the bromocriptine treatment. In explaining this result, one has to keep in mind that: 1) T4 has a large intrathyroidal pool (Chanoine et al., 1993); 2) T4 half-life in the serum is considerably longer than that of T3 (Unger et al., 1980); and 3) The T4–T3 conversion is inhibited (Johnson et al., 2003). Therefore, it is conceivable to speculate that a

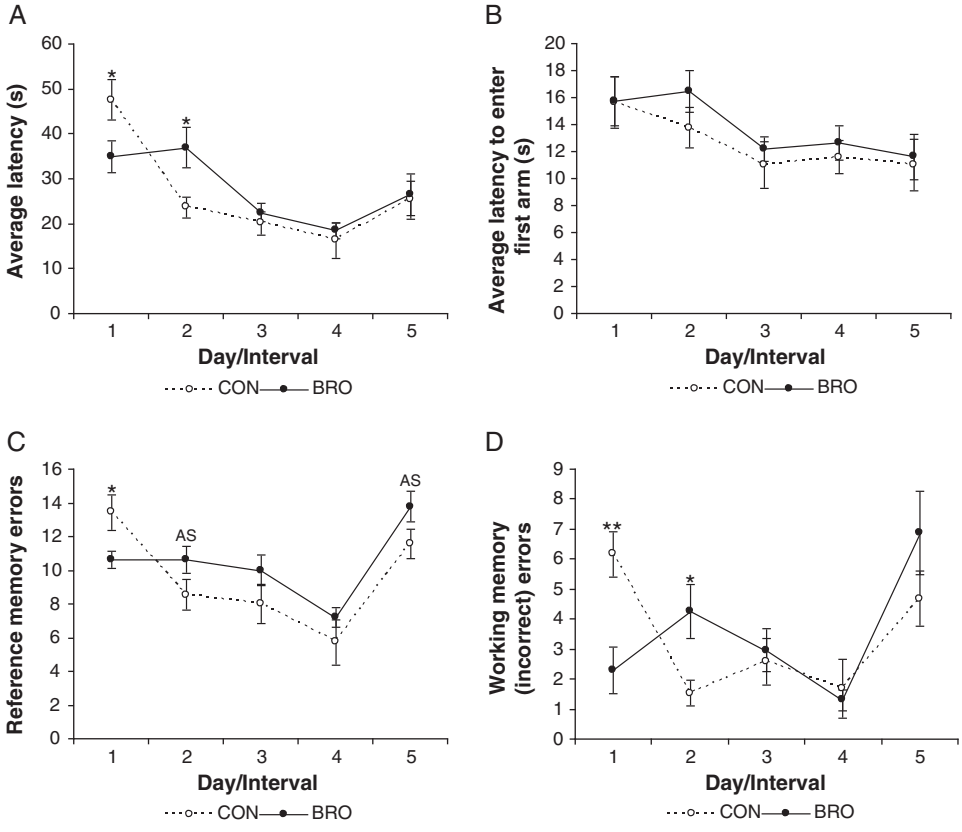

Fig. 1. Memory/learning performance as measured in the radial arm water maze. BRO animals presented a much less accentuated improvement in performance from the 1st to the 4th testing day (A). This result is associated with a higher number of reference memory errors (C) and higher number of working memory (incorrect) errors (D), but does not seem to be related with the latency to enter the first arm (B). Values are means ± SEM. AS $0.10<P<0.05$, *$P<0.05$, and **$P<0.01$.

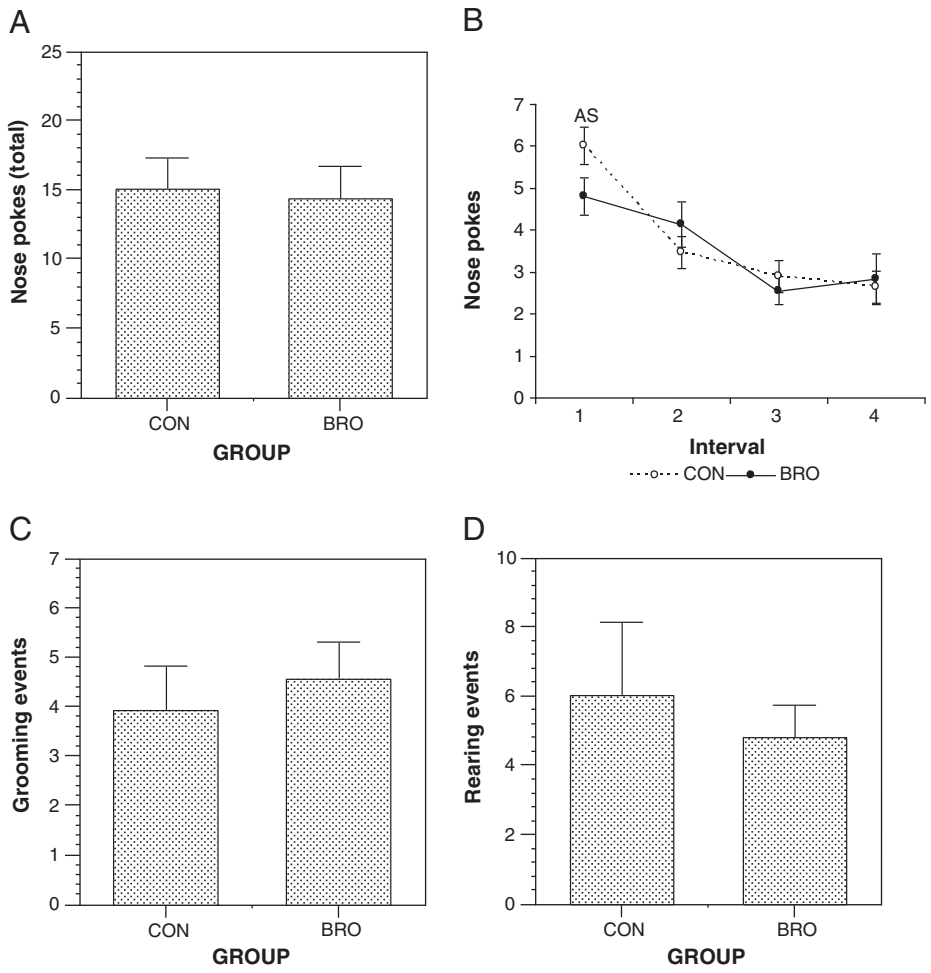

Fig. 2. Novelty-seeking behavior as measured by the number of nose pokes in the hole board (HB) arena. No difference was observed between bromocriptine (BRO) and control (CON) rats regarding the total number of nose pokes (A). When intra-session intervals were considered, a difference approaching significance was observed for the first interval ($P = 0.055$ — B). Motor activity, as measured by the number of grooming (C) and rearing (D) events in the HB, was also not affected by BRO treatment. Values are means ± SEM. AS $0.10 < P < 0.05$.

longer period of malnourishment would be needed to cause a detectable reduction in serum T4 levels.

4.2. Behavior

Our main findings indicate that maternal hypoprolactinemia induced by bromocriptine treatment during the late lactating period causes behavioral changes in the adult offspring. In the radial arm water maze, BRO rats had a less pronounced gain in performance from the 1st to the 4th testing day than CON ones, indicating that the former had significant alterations in visuospatial memory/learning mechanisms. In spite of the fact that the performance gain was more evident in CON animals, a peculiar finding was that BRO offspring were faster than CON ones in locating the escape platform during the 1st testing day. The latencies to enter for the first time any of the arms were equivalent in both groups throughout the testing sessions; therefore performance differences in the first testing day cannot be attributed to a delay in the decision to start moving and exploring the maze. Furthermore, the number of working memory errors of the correct type (as measured by the number of re-entries into the arm in which the platform was located) was very low and similar between groups, indicating that most animals, irrespective of treatment, upon entering the correct arm almost always found the platform and successfully exited the water. On the other hand, during the 1st day, BRO animals made significantly less reference memory errors (−21%, as measured by the number of first entries into arms that did not have the platform) and considerably less working memory errors of the incorrect type (−63%, as measured by the number of re-entries into arms that did not have the platform) than CON rats. This pattern of results suggests that the working memory of BRO rats is not as affected as that of CON rats by the initial contact with the RAWM, leading to a shorter path length to find the platform and the consequent reduced latency. By the second testing day, the initial BRO advantage is lost mainly due to a marked reduction in working memory errors of the incorrect type by CON rats, an improvement in performance that was completely absent in BRO offspring.

As for the EPM, our results indicated that BRO offspring displayed more intense anxious-like behavior, characterized by a shorter time spent in and reduced number of entries into the open arms. These animals also spent less time in the central area of the EPM, which has been associated with decision making processes (Johnson and Rodgers, 1996) and had a reduced number of closed arm entries, indicative of lowered overall locomotor activity. No differences were observed regarding the ethologically derived measures associated with risk assessment, vertical activity and exploration (Rodgers et al., 1997), suggesting that the effects of BRO programming on behavior were selective.

Both groups displayed a similar level of novelty-seeking behavior in the HB. Furthermore, the analyses of the number of grooming and rearing events in the HB also failed to indicate any differences between groups. The absence of differences between groups regarding these behavioral traits in the HB gives further support to the

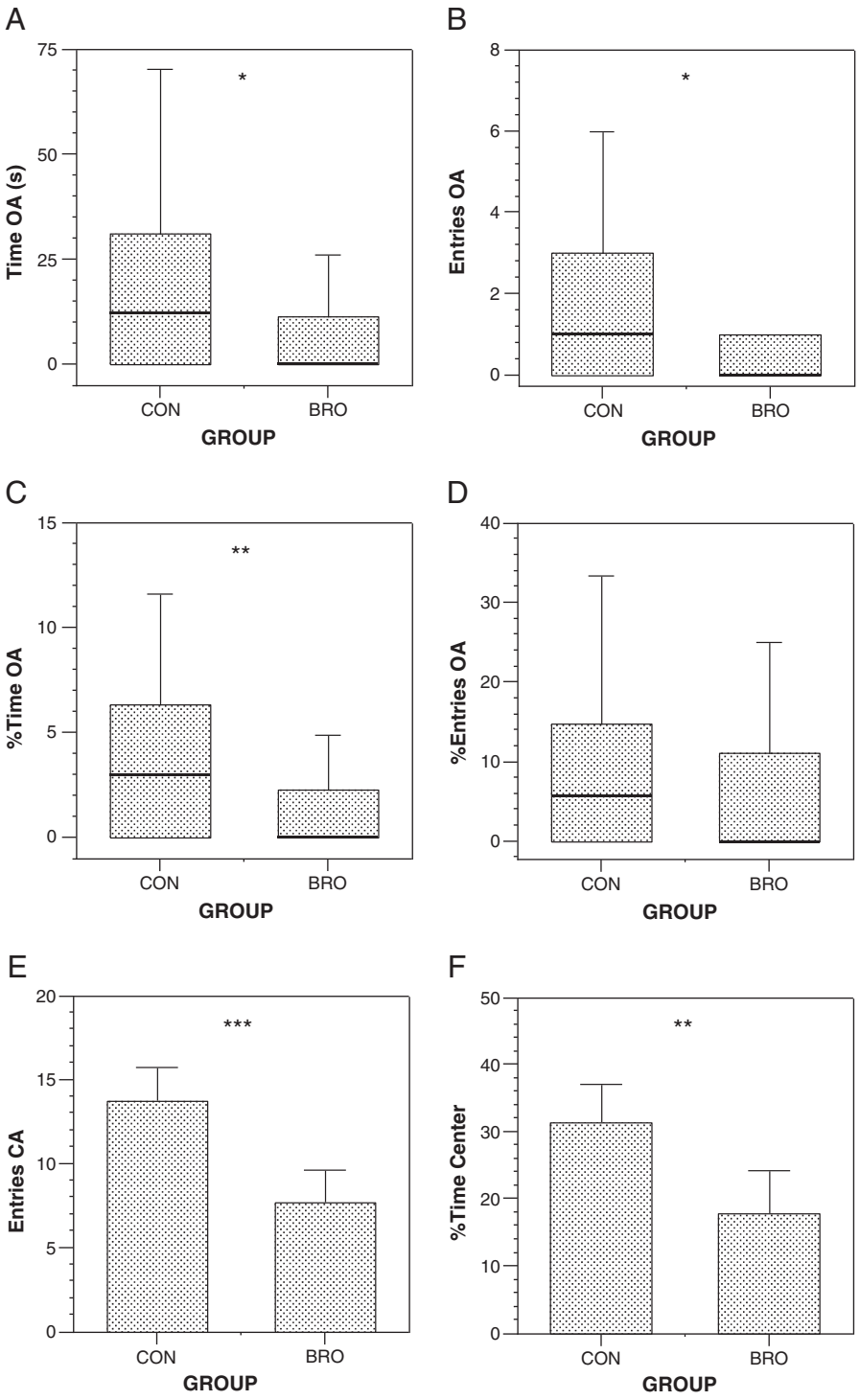

Fig. 3. Anxiety-like behavior tested in the elevated plus maze. Bromocriptine (BRO) rats had significantly lower Time OA (A), Entries OA (B) and %Time OA (C) than control (CON) ones, indicative of higher anxiety levels. Locomotor activity (Entries CA — E) and decision making (%Time Center — F) were also reduced in BRO animals. A, B, C, and D: values are medians ± quartile, E and F: values are means ± SEM, *P<0.05, **P<0.01, and ***P<0.001.

notion that the effects of BRO programming on behavior were selective.

In summary, a three-day period of malnutrition resulting from the inhibition of milk production by bromocriptine treatment of dams at the end of lactation affected learning and memory mechanisms and increased anxiety-like behavioral traits in adult Wistar rats without modifying novelty-seeking behavior. In addressing possible explanations for the present results, two lines of reasoning, which are not mutually exclusive, may be used: 1) the altered behaviors were directly programmed during the period of malnourishment at the end of lactation, and/or 2) the behavioral changes are a consequence of the metabolic/hormonal changes programmed during the lactation period.

4.3. Malnutrition, brain development and behavior

Events that affect the central nervous system (CNS) during sensitive periods of its development may result in long-lasting

changes in the formation and function of brain circuits (Bhatnagar et al., 2005; Charney and Bremner, 1999) and could increase the chance of developing cognitive deficits at adulthood (Charney and Bremner, 1999; Strupp and Levitsky, 1995). Malnourished children have profound changes on brain development such as disorderly differentiation, decreased number of synapses and synaptic neurotransmitter content, delayed myelination and reduced overall development of dendritic arborization (Udani, 1992; Wang and Brand-Miller, 2003). The important changes in the temporal sequence of brain maturation associated with malnutrition permanently impair cognitive functions (Strupp and Levitsky, 1995). For instance, cognitive flexibility, attention, working memory, visual perception and memory performance are affected by child malnutrition (Kar et al., 2008).

In rodents, a period of rapid brain growth begins prenatally, mainly during the last week of gestation, and peaks during lactation (Andrade et al., 1996). There are studies in rats indicating that the postnatal period should be susceptible to the deleterious effects of malnutrition on brain function because of its rapid growth during this period (Dobbing, 1964; Winick and Noble, 1966). In particular, the first 3 weeks of postnatal life correspond to a time-window of intense hippocampal and hypothalamic neuronal circuit development (Bouret et al., 2004; Coupé et al., 2009), suggesting that early perinatal modifications may have long-lasting consequences on behavior and neuroendocrine parameters (Bunsey and Eichenbaum, 1995, 1996). In fact, animal studies have shown that early malnutrition permanently affects brain structure (Jahnke and Bedi, 2007; Morgane et al., 1993; Randt and Derby, 1973) as well as behavior (Levay et al., 2008; Pereira-da-Silva et al., 2009). Alterations in hippocampal development and function as a result of malnutrition (Jahnke and Bedi, 2007; Levitsky and Strupp, 1995) are consistent with our current findings of impaired performance gain in the RAWM and higher anxiety levels in the EPM displayed by BRO rats, since the hippocampus has an important role in normal memory processing (O'Keefe, 1990; Olton et al., 1978), locomotor activity and anxiety-like behavior (Bannerman et al., 2004; Bast and Feldon, 2003). Compounding the effects of malnutrition on the structural development of the hippocampus is the fact that this structure is also particularly sensitive to anxiogenic factors (Ciaroni et al., 2002). Malnutrition is a particularly stressful event (Yehuda et al., 2009), an observation that is in agreement with our finding of increased corticosterone levels in BRO animals at PN21. Malnourishment is not only a stressful factor that affects learning/performance but also programs for anxiety (Levitsky and Barnes, 1970) and changes in decision making (Taylor and MacQueen, 2007). Our results complement previous findings in the sense that we demonstrated that even restricted periods of malnourishment resulting from a premature interruption of the lactation period has long lasting behavioral effects.

Considering that bromocriptine is an agonist of the type 2 dopaminergic receptor, it is not inconceivable that this substance could have affected the dams' maternal behavior, which, in turn, could have had long lasting effects on offspring behavior. Bromocriptine could affect maternal behavior directly, by altering dopaminergic function (Koller, 1992), or indirectly via reduction of PRL levels. PRL is associated with the onset of maternal behavior in rats (Mann and Bridges, 2001). Furthermore, PRL can have antidepressive and anxiolytic effects, reducing HPA axis responses to stress in the rat (Drago et al., 1990; Frye and Walf, 2004; Lund et al., 2005; Torner et al., 2002). Since bromocriptine treatment did not have a significant effect on the dams' corticosterone levels and since PRL has not been implicated in the continuation of maternal behavior, particularly at the end of the lactation period, an explanation for our results based on behavioral alterations in the dams is rendered unlikely.

Early stressors may have a permanent rather than transient effect on the organism (Darnaudéry and Maccari, 2008). Early stress leads to long-term consequences from both behavioral and neurobiological points of view, increasing susceptibility to anxiety- and depression-like behavior and memory impairment in the offspring (Charney and Bremner, 1999; Darnaudéry and Maccari, 2008; Vallée et al., 1999). Among the mechanisms involved in these behavioral alterations are increased responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to stress and the endocrine stress response (Henry et al., 1994; Morley-Fletcher et al., 2003a,b; Vallée et al., 1997), which is accompanied by reduced hippocampal plasticity (Darnaudéry and Maccari, 2008). During neonatal life, psychological stress has been associated with an increase in corticotropin releasing hormone (CRH) expression in the amygdala and hippocampus (Hatalski et al., 2000), which also may exert a role in the long-lasting effects of early-life stress (Brunson et al., 2001).

Thyroid hormones also affect several processes in brain development during different time windows (Anderson, 2001; Bernal, 2002; Zoeller and Rovet, 2004). Experimental studies in rat have firmly established that thyroid deficiency during the early postnatal period markedly impairs the development of the CNS and delays the maturation of innate reflexes and behavior (Eayrs, 1964; Legrand, 1983; Meisami et al., 1992). Some of the most prominent actions of thyroid hormones occur on 2rd and 3rd week postnatal (Dussault and Ruel, 1987; Porterfield and Hendrich, 1993; Thompson and Potter, 2000). In the rat, several aspects of adaptive, cognitive and motor development, which are believed to be related to hippocampal development (Korányi et al., 1976; Leblanc and Bland, 1979), can be impaired in the presence of postnatal hypothyroidism (Tamasy et al., 1986a,b; Hasegawa et al., 2010).

4.4. Endocrine parameters at adulthood and behavior

The programming model used in the present study has several characteristics that can increase the complexity of the discussion of our results. For instance, we have previously demonstrated that BRO offspring, as adults, present higher central adiposity, higher triglycerides, lower HDL-c, insulin resistance (De Moura et al., 2009), hypothyroidism (Bonomo et al., 2008), hypercorticosteronemia, higher adrenal catecholamine content (De Moura et al., 2009), hyperleptinemia and resistance to the anorexigenic effect of leptin (Bonomo et al., 2007). These characteristics, by themselves or in conjunction, are known to interfere with behavior (Farr et al., 2008; Fraga-Marques et al., 2009, 2010; Harvey and Ashford, 2003; Kamal et al., 1999).

Cognitive deficits have already been evidenced in obese rodents with associated hypertriglyceridemia (Farr et al., 2008) and rodents with diabetes (Harvey and Ashford, 2003; Kamal et al., 1999). As another example, we have recently shown that rats that were programmed for hyperleptinemia and resistance to the anorexigenic effect of leptin at adulthood had significant behavioral alteration in the RAWM, EPM and HB (Fraga-Marques et al., 2009; 2010). Concerning the effects of elevated corticosterone levels at adulthood, it has been shown that infusion of glucocorticoids into the amygdala can increase anxiety and also further increase glucocorticoids secretion (Myers and Greenwood-Van Meerveld, 2007; Shepard et al., 2000, 2003). We have also previously shown that rats with higher serum corticosterone levels at adulthood display increased anxiety-like behavior in the EPM and impaired learning/memory performance in the RAWM (Fraga-Marques et al., 2009). As for hypothyroidism at adulthood, several studies have already shown that the thyroid status is linked with anxiety-like behavior and impaired learning in rodents (Guadaño-Ferraz et al., 2003; Pilhatsch et al., 2010; Venero et al., 2005) and more specifically with decreased locomotor activity in the EPM (Fundaro, 1989; Redei et al., 2001).

5. Conclusions

Our data indicate that milk inhibition at the end of lactation caused by maternal treatment with bromocriptine results in hypercorticosteronemia and hypothyroidism at the end of the period of restricted

nutrition. We further demonstrated that bromocriptine treatment resulted in altered learning/memory performance and higher levels of anxiety-like behavior, without interfering with novelty-seeking behavior, in the adult offspring. Subsequent studies will have to address whether corrected to normal parameters regarding HPA axis and thyroid status can improve the observed behavioral alterations or if behavioral modifications are a permanent effect of altered brain development and function resulting from early malnutrition. The present study suggests that the experimental model used here will be useful to address the effects of shortened periods of lactation, a condition that is becoming increasingly more prevalent in modern society.

Acknowledgments

Funding for this study was provided by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and by Sub-Reitoria de Pós-graduação e Pesquisa da Universidade do Estado do Rio de Janeiro (SR2-UERJ). MCF and ITB are recipients of FAPERJ postdoctoral fellowships, while JOS is recipient of CNPq fellowship for undergraduate students. The authors are thankful to Ulisses Risso Siqueira for the animal care.

References

Abreu-Villaça Y, Queiroz-Gomes F, Do E, Dal Monte AP, Filgueiras CC, Manhães AC. Individual differences in novelty-seeking behaviour but not in anxiety response to a new environment can predict nicotine consumption in adolescent C57BL/6 mice. Behav Brain Res 2006;167:175–82.

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250–2.

Anderson GW. Thyroid hormones and the brain. Front Neuroendocrinol 2001;22:1–17.

Andrade JP, Castanheira-Vale AJ, Paz-Dias PG, Madeira MD, Paula-Barbosa MM. The dendritic trees of neurons from the hippocampal formation of protein deprived adult rats. A quantitative Golgi study. Exp Brain Res 1996;109:419–33.

Armitage JA, Poston L, Taylor PD. Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. Front Horm Res 2008;36:73–84.

Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. Regional dissociations within the hippocampus-memory and anxiety. Neurosci Biobehav Rev 2004;28:273–83.

Barker DJ. The developmental origins of adult disease. Eur J Epidemiol 2003;18:733–6.

Bast T, Feldon J. Hippocampal modulation of sensorimotor processes. Prog Neurobiol 2003;70:319–45.

Bernal J. Action of thyroid hormone in brain. J Endocrinol Invest 2002;25:268–88.

Bhatnagar S, Lee TM, Vining C. Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats. Horm Behav 2005;47:430–8.

Bonomo IT, Lisboa PC, Passos MC, Pazos-Moura CC, Reis AM, Moura EG. Prolactin inhibition in lactating rats changes leptin transfer through the milk. Horm Metab Res 2005;37:220–5.

Bonomo IT, Lisboa PC, Pereira AR, Passos MC, de Moura EG. Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring. J Endocrinol 2007;192:339–44.

Bonomo IT, Lisboa PC, Passos MC, Alves SB, Reis AM, de Moura EG. Prolactin inhibition at the end of lactation programs for a central hypothyroidism in adult rat. J Endocrinol 2008;198:331–7.

Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 2004;304:108–10.

Brunson KL, Avishai-Eliner S, Hatalski CG, Baram TZ. Neurobiology of the stress response early in life: evolution of a concept and the role of corticotropin releasing hormone. Mol Psychiatry 2001;6:647–56.

Bunsey M, Eichenbaum H. Selective damage to the hippocampal region blocks long-term retention of a natural and nonspatial stimulus–stimulus association. Hippocampus 1995;5:546–56.

Bunsey M, Eichenbaum H. Conservation of hippocampal memory function in rats and humans. Nature 1996;379:255–7.

Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res 2002;134:49–57.

Chanoine JP, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S, et al. The thyroid gland is a major source of circulating T3 in the rat. J Clin Invest 1993;91:2709–13.

Charney DS, Bremner JD. The neurobiology of anxiety disorders. In: Charney DS, Nestler EJ, Bunney BS, editors. The neurobiology of mental illness. New York: Oxford University Press; 1999. p. 494–517.

Ciaroni S, Cecchini T, Ferri P, Cuppini R, Ambrogini P, Santi S, et al. Neural precursor proliferation and newborn cell survival in the adult rat dentate gyrus are affected by vitamin E deficiency. Neurosci Res 2002;44:369–77.

Coupé B, Dutriez-Casteloot I, Breton C, Lefèvre F, Mairesse J, Dickes-Coopman A, et al. Perinatal undernutrition modifies cell proliferation and brain-derived neurotrophic factor levels during critical time-windows for hypothalamic and hippocampal development in the male rat. J Neuroendocrinol 2009;21:40–8.

Darnaudéry M, Maccari S. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res Rev 2008;57:571–85.

De Moura EG, Lisboa PC, Passos MC. Neonatal programming of neuroimmunomodulation-role of adipocytokines and neuropeptides. Neuroimmunomodulation 2008;15:176–88.

De Moura EG, Bonomo IT, Nogueira-Neto JF, de Oliveira E, Trevenzoli IH, Reis AM, et al. Maternal prolactin inhibition during lactation programs for metabolic syndrome in adult progeny. J Physiol 2009;587:4919–29.

Dobbing J. The influence of early nutrition on the development and myelination of the brain. Proc R Soc Lond B Biol Sci 1964;159:503–9.

Drago F, Pulvirenti L, Spadaro F, Pennisi G. Effects of TRH and prolactin in the behavioral despair (swim) model of depression in rats. Psychoneuroendocrinology 1990;15:349–56.

Dussault JH, Ruel J. Thyroid hormones and brain development. Annu Rev Physiol 1987;49:321–34.

Eayrs JT. Endocrine influence on cerebral development. Arch Biol (Liege) 1964;75:529–65.

Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, et al. Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 2008;149:2628–36.

Fischbeck KL, Rasmussen KM. Effect of repeated reproductive cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. J Nutr 1987;117:1967–75.

Fraga-Marques MC, Moura EG, Claudio-Neto S, Trevenzoli IH, Toste FP, Passos MCF, et al. Neonatal hyperleptinaemia programmes anxiety-like and novelty seeking behaviours but not memory/learning in adult rats. Horm Behav 2009;55:272–9.

Fraga-Marques MC, Moura EG, Silva JO, Claudio-Neto S, Pereira-Toste F, Passos MC, et al. Effects of maternal hyperleptinaemia during lactation on short-term memory/learning, anxiety-like and novelty-seeking behavioral traits of adult male rats. Behav Brain Res 2010;206:147–50.

Frye CA, Walf AA. Hippocampal 3alpha, 5alpha-THP may alter depressive behavior of pregnant and lactating rats. Pharmacol Biochem Behav 2004;78:531–40.

Fundaro A. Behavioral modifications in relation to hypothyroidism and hyperthyroidism in adult rats. Prog Neuropsychopharmacol Biol Psychiatry 1989;13(6):927–40.

Galtry J. Lactation and the labor market: breastfeeding, labor market changes, and public policy in the United States. Health Care Women Int 1997;18:467–80.

Gluckman PD, Hanson MA. Developmental plasticity and human disease: research directions. J Intern Med 2007;261:461–71.

Gomendio M, Cassinello J, Smith MW, Bateson P. Maternal state affects intestinal changes of rat pups at weaning. Behav Ecol Sociobiol 1995;37:71–80.

Guadaño-Ferraz A, Benavides-Piccione R, Venero C, Lancha C, Vennström B, Sandi C, et al. Lack of thyroid hormone receptor alpha1 is associated with selective alterations in behavior and hippocampal circuits. Mol Psychiatry 2003;8:30–8.

Handley SL, McBlane JW. An assessment of the elevated x-maze for studying anxiety and anxiety-modulating drugs. J Pharmacol Toxicol Methods 1993;29:129–38.

Harvey J, Ashford ML. Leptin in the CNS: much more than a satiety signal. Neuropharmacology 2003;44:845–54.

Hasegawa M, Kida I, Wada H. A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid. Neurosci Lett 2010;479:240–4.

Hatalski CG, Brunson KL, Tantayanubutr B, Chen Y, Baram TZ. Neuronal activity and stress differentially regulate hippocampal and hypothalamic corticotropin-releasing hormone expression in the immature rat. Neuroscience 2000;101(3):571–80.

Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab 2009;23:793–800.

Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. J Neuroendocrinol 1994;6:341–5.

Huynh H, Feldt LS. Estimation of the Box correction for degrees of freedom from the sample data in randomized block and split-plot designs. J Educ Stat 1976;1:69–82.

Jahnke S, Bedi KS. Undernutrition during early life increases the level of apoptosis in the dentate gyrus but not in the CA2 + CA3 region of the hippocampal formation. Brain Res 2007;1143:60–9.

Jarrard LE. On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 1993;60:9–26.

Johnson NJ, Rodgers RJ. Ethological analysis of cholecystokinin (CCKA and CCKB) receptor ligands in the elevated plus-maze test of anxiety in mice. Psychopharmacology (Berl) 1996;124:355–64.

Johnson PJ, Messer IV NT, Ganjam VK, Thompson Jr DL, Refsal KR, Loch WE, et al. Effects of propylthiouracil and bromocryptine on serum concentrations of thyrotrophin and thyroid hormones in normal female horses. Equine Vet J 2003;35:296–301.

Kamal A, Spoelstra K, Biessels GJ, Urban IJ, Gispen WH. Effects of changes in glucose concentration on synaptic plasticity in hippocampal slices. Brain Res 1999;824:238–42.

Kar BR, Rao SL, Chandramouli BA. Cognitive development in children with chronic protein energy malnutrition. Behav Brain Funct 2008;4:31.

Koller WC. Initiating treatment of Parkinson's disease. Neurology 1992;42:33–8.

Korányi L, Tamásy V, Lissák K, Király I, Borsy J. Effect of thyrotropin-releasing hormone (TRH) and antidepressant agents on brain stem and hypothalamic multiple unit activity in the cat. Psychopharmacology (Berl) 1976;49:197–200.

Leblanc MO, Bland BH. Developmental aspects of hippocampal electrical activity and motor behavior in the rat. Exp Neurol 1979;66:220–37.

Legrand J. Thyroid hormones and maturation of the nervous system. J Physiol Paris 1983;78:603–52.

Levay EA, Paolini AG, Govic A, Hazi A, Penman J, Kent S. Anxiety-like behaviour in adult rats perinatally exposed to maternal calorie restriction. Behav Brain Res 2008;191: 164–72.
Levitsky DA, Barnes RH. Effect of early malnutrition on the reaction of adult rats to aversive stimuli. Nature 1970;225:468–9.
Levitsky DA, Strupp BJ. Malnutrition and the brain: changing concepts, changing concerns. J Nutr 1995;125:2212S–20S.
Lisboa PC, Passos MC, Dutra SC, Bonomo IT, Denolato AT, Reis AM, et al. Leptin and prolactin, but not corticosterone, modulate body weight and thyroid function in protein-malnourished lactating rats. Horm Metab Res 2006;38:295–9.
Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 1998;317:1481–7.
Lund TD, Rovis T, Chung WC, Handa RJ. Novel actions of estrogen receptor-beta on anxiety-related behaviors. Endocrinology 2005;146:797–807.
Manhães AC, Guthierrez MCS, Filgueiras CC, Abreu-Villaça Y. Anxiety-like behavior during nicotine withdrawal predict subsequent nicotine consumption in adolescent C57BL/6 mice. Behav Brain Res 2008;193:216–24.
Mann PE, Bridges RS. Lactogenic hormone regulation of maternal behavior. Prog Brain Res 2001;133:251–62.
Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. Acta Cir Bras 2009;24:69–74.
Meisami E, Sendera TJ, Clay LB. Paradoxical hypertrophy and plasticity of the testis in rats recovering from early thyroid deficiency: a growth study including effects of age and duration of hypothyroidism. J Endocrinol 1992;135:495–505.
Molina VA, Keller EA, Orsingher OA. Reduced anti-immobility effect of repeated desipramine (DMI) treatment in adult rats undernourished at perinatal age. Pharmacol Biochem Behav 1987;26:417–9.
Morgane PJ, Austin-LaFrance R, Bronzino J, Tonkiss J, Díaz-Cintra S, Cintra L, et al. Prenatal malnutrition and development of the brain. Neurosci Biobehav Rev 1993;17:91–128. Spring.
Morley-Fletcher S, Rea M, Maccari S, Laviola G. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur J Neurosci 2003a;18:3367–74.
Morley-Fletcher S, Darnaudery M, Koehl M, Casolini P, Van Reeth O, Maccari S. Prenatal stress in rats predicts immobility behavior in the forced swim test. Effects of a chronic treatment with tianeptine. Brain Res 2003b;989:246–51.
Moura EG, Ramos CF, Nascimento CC, Rosenthal D, Breitenbach MM. Thyroid function in fasting rats: variations in ^{131}I uptake and transient decrease in peroxidase activity. Braz J Med Biol Res 1987;20:407–10.
Myers B, Greenwood-Van Meerveld B. Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. Am J Physiol Gastrointest Liver Physiol 2007;292:1622–9.
O'Keefe J. A computational theory of the hippocampal cognitive map. Prog Brain Res 1990;83:301–12.
Olton DS, Walker JA, Gage FH. Hippocampal connections and spatial discrimination. Brain Res 1978;139:295–308.
Passos MCF, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. Nutr Res 2000;20: 1603–12.
Pereira-da-Silva MS, Cabral-Filho JE, de-Oliveira LM. Effect of early malnutrition and environmental stimulation in the performance of rats in the elevated plus maze. Behav Brain Res 2009;205:286–9.
Pilhatsch M, Winter C, Nordström K, Vennström B, Bauer M, Juckel G. Increased depressive behaviour in mice harboring the mutant thyroid hormone receptor alpha 1. Behav Brain Res 2010;214:187–92.
Plagemann A, Harder T. Breast feeding and the risk of obesity and related metabolic diseases in the child. Metab Syndr Relat Disord 2005;3:222–32.
Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development-current perspectives. Endocr Rev 1993;14:94–106.
Randt CT, Derby BM. Behavioral and brain correlations in early life nutritional deprivation. Arch Neurol 1973;28:167–72.
Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 1976;295:349–53.
Redei EE, Solberg LC, Kluczynski JM, Pare WP. Paradoxical hormonal and behavioral responses to hypothyroid and hyperthyroid states in the Wistar-Kyoto rat. Neuropsychopharmacology 2001;24:632–9.
Rodgers B. Feeding in infancy and later ability and attainment: a longitudinal study. Dev Med Child Neurol 1978;20:421–6.
Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997;30:289–304.
Shah GV, Shyr SW, Grosvenor CE, Crowley WR. Hyperprolactinemia after neonatal prolactin (PRL) deficiency in rats: evidence for altered anterior pituitary regulation of PRL secretion. Endocrinology 1988;122:1883–9.
Shepard JD, Barron KW, Myers DA. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res 2000;861:288–95.
Shepard JD, Barron KW, Myers DA. Stereotaxic localization of corticosterone to the amygdala enhances hypothalamo-pituitary-adrenal responses to behavioral stress. Brain Res 2003;963:203–13.
Strupp BJ, Levitsky DA. Enduring cognitive effects of early malnutrition: a theoretical reappraisal. J Nutr 1995;125:2221S–32S.
Tamasy V, Meisami E, Vallerga A, Timiras PS. Rehabilitation from neonatal hypothyroidism: spontaneous motor activity, exploratory behavior, avoidance learning and responses of pituitary-thyroid axis to stress in male rats. Psychoneuroendocrinology 1986a;11:91–103.
Tamasy V, Meisami E, Du JZ, Timiras PS. Exploratory behavior, learning ability, and thyroid hormonal responses to stress in female rats rehabilitating from postnatal hypothyroidism. Dev Psychobiol 1986b;19:537–53.
Taylor VH, MacQueen GM. Cognitive dysfunction associated with metabolic syndrome. Obes Rev 2007;8:409–18.
Thompson CC, Potter GB. Thyroid hormone action in neural development. Cereb Cortex 2000;10:939–45.
Torner L, Toschi N, Nava G, Clapp C, Neumann ID. Increased hypothalamic expression of prolactin in lactation: involvement in behavioural and neuroendocrine stress responses. Eur J Neurosci 2002;15:1381–9.
Udani PM. Protein energy malnutrition (PEM), brain and various facets of child development. Indian J Pediatr 1992;59:165–86.
Unger J, Van Heuverswyn B, Decoster C, Cantraine F, Mockel J, Van Herle A. Thyroglobulin and thyroid hormone release after intravenous administration of bovine thyrotropin in man. J Clin Endocrinol Metab 1980;51:590–4.
Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 1997;17:2626–36.
Valzelli L, Garattini S. Biochemical and behavioural changes induced by isolation in rats. Neuropharmacology 1972;11:17–22.
Venero C, Guadaño-Ferraz A, Herrero AI, Nordström K, Manzano J, de Escobar GM, et al. Anxiety, memory impairment, and locomotor dysfunction caused by a mutant thyroid hormone receptor alpha1 can be ameliorated by T3 treatment. Genes Dev 2005;19:2152–63.
Wang B, Brand-Miller J. The role and potential of sialic acid in human nutrition. Eur J Clin Nutr 2003;57:1351–69.
Winick M, Noble A. Cellular response in rats during malnutrition at various ages. J Nutr 1966;89:300–6.
Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. J Psychiatr Res 2009;43:877–83.
Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol 2004;16:809–18.